molecular mass information and a combined peak table (base peak masses assigned to ELSD peaks). The decision process is based on the analysis of purity (% ELSD area), assignable base peak mass, the number of peaks with the same base peaks (to account for isomers) and minimum relative and minimum absolute peak area of the ELSD signal. An additional criterion which can be applied is the number of fractions one wants to collect for the preparative run.

To demonstrate the decision power of the algorithm a copy of a result file of a representative subset of a library of 192 isoxazoles prepared by solid-phase synthesis[12] and analyzed by analytical LC/MS ELSD is shown (Table 1). It consists of several columns which read out general result information, mass information for triggering preparative fraction collection (mixtures only) and as a quality measure the sum of the ELSD area for the number of peaks used for fraction collection. Analysis of this result file in a spreadsheet program can now efficiently guide the decision process for sample follow-up. For comparison purposes the ELSD signal and the total ion current (TIC) of typical examples of the 'pure known' (Fig 2) and the 'mixture with known compound' (Fig 3) category are shown.

In summary, a post-synthesis data evaluation process has been established which allows us to cope intelligently with the large data sets obtained by analysis of libraries from parallel synthesis efforts. It relies on the analysis of purity as well as on identification criteria and efficiently limits the compounds submitted to screening to manageable and traceable numbers. Contrary to purification efforts, which only focus on the isolation of expected compounds,[13] we think that novel compounds should not be thrown away simply because they are of unknown structure but be used as a source of serendipity in the lead-finding process.

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Commercial development and introduction of DiTera™, a new nematicide

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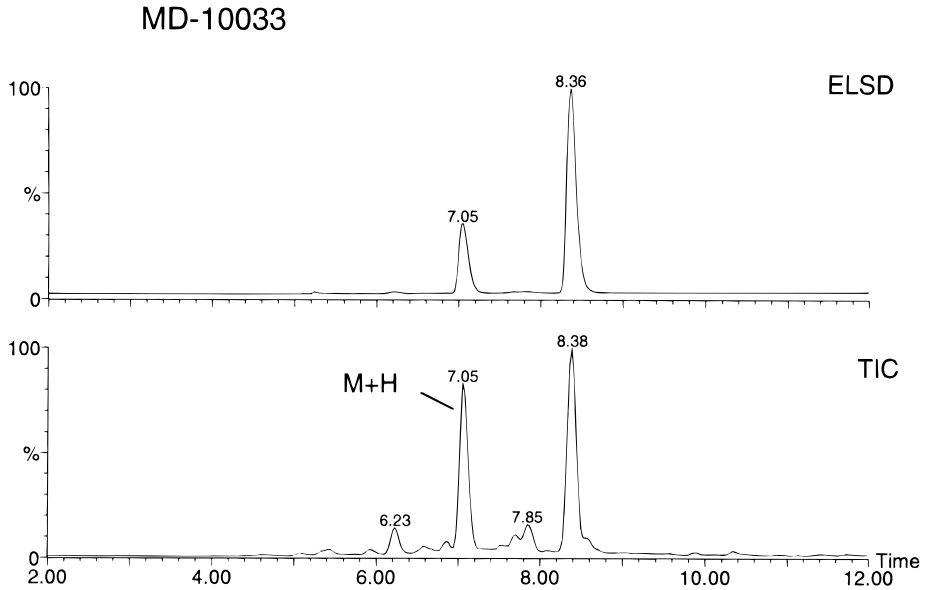

Figure 3. ELSD and total ion control of a mixture with known compound from Table 1.

Abstract: DiTera, a new biological nematicide derived from the fermentation of a nematode-parasitic isolate of fungi of *Myrothecium* spp, was recently introduced in selected markets in North America. The product and its formulations have been registered as a microbial nematicide in several countries. Toxicological studies indicated a very favourable acute and non-target toxicity profile and suggest minimal adverse effects on non-target organisms. Field evaluations of DiTera on turf, bananas, and field, fruit and vegetable crops such as tobacco, grapes, citrus and cole crops indicate a significant reduction in populations of the major nematode pests affecting those crops, including root-knot, cyst, sting and burrowing nematodes, during the critical stages of plant growth. An overall increase in yield comparable to that obtained with the current chemical standards was obtained in the majority of the studies. This product thus has a unique profile presenting a new concept in microbial pesticide development and provides a new option for integrated management of nematode pests.

Keywords: DiTera; nematicide; Myrothecium; natural product; biological control; ABG-9008

1 INTRODUCTION

Plant parasitic nematodes cause significant damage to agricultural crops worldwide. Economic loss due to these microscopic pathogens was estimated at over US$ 100 billion at the global level.[1] The yield loss on selected life-sustaining and economically important crops alone was reported at approximately 12.3%. This yield reduction is due to feeding on the vascular tissues in the roots, depriving plants of essential nutrients; nematodes have also been implicated in disease complexes and as transmitters of viral diseases on specific crops. Chemical nematicides of the organophosphate or carbamate groups are by far the most widely used means of combating soil and plant nematodes; however, increasing awareness of potential problems with residues of currently used chemical products has resulted in an extensive search for and development of environmentally compatible products specifically affecting nematodes. More recently, concerns on depletion of ozone layers in the stratosphere,[2] implicating the soil fumigant methyl bromide, has accelerated the search for newer means to combat these soil-dwelling plant pathogens. Use of biological control methods involving fungal or bacterial parasites of nematodes, though attempted with success in several laboratory and greenhouse situations, has rarely been realized into commercial products. The primary reason for this is believed to be difficulties in manufacturing, quality control, and the variability in actual field efficacy of these products.

DiTera is a product of biological origin, produced by the fermentation of a strain of the soil hyphomycete fungus, *Myrothecium verrucaria* (Alb & Schw) Ditm ex Steudel. Gintis *et al*[3] reported the isolation of strains of *M verrucaria* associated with cysts of the soybean cyst nematode, *Heterodera glycines* Ichinohe. Although originally isolated from nematode cadavers, early greenhouse evaluations used live cultures (Rodriguez-Kabana, R unpublished), but did not demonstrate adequate control of plant nematodes. However, selection of sub-isolates (AARC-0255), fermentation improvements, and biological evaluations with extracted preparations of the fungal fermentation, provided significant reduction of galling by root-knot nematodes, and mortality of other ecto-parasitic nematodes in laboratory and greenhouse and bioassays.[4] Further fermentation improvements and process modifications resulted in the development of a technical grade active ingredient for a product coded as ABG-9008, later to be commercialized under the trade name DiTera.

M verrucaria is cosmopolitan in distribution and several isolates have been reported as a root pathogen on red clover and alfalfa[5] and as leaf and stem pathogens of several economically important crops. The fungus has been well described by Domsch *et al*.[6] Except for the isolated report on the isolation of the above mentioned strain, *M verrucaria* has not been associated with nematodes.

2 BIOLOGICAL ACTIVITY

The development of DiTera resulted from its specific nematicidal activity against second-stage juveniles of the root-knot nematode, *Meloidogyne incognita* (Kof & White) Chitwood, in a target-directed contact assay which measured the motility responses of the nematodes on incubation in solutions of fungal extracts over a defined period of time (24 or 48 h). Irreversibility of the response in the non-motile nematodes was confirmed by transferring the DiTera-treated specimen into deionized water and observing its behaviour. Increasing concentrations of fungal extracts resulted in a faster rate of mortality. Separation of constituents of mycelial and fermentation broths, followed by testing of a mycelial extract in acetone + water (1 + 1 by volume) solutions suggested the component(s) with biological activity to be highly polar; the activity was also found to be extremely stable to physical factors such as heat and pH. Even though most of the initial studies were focused on *M incognita* juveniles, studies at Abbott Laboratories and elsewhere have confirmed the activity against several additional plant parasitic nematode species such as citrus (*Tylenchulus semipenetrans* Cobb), burrowing (*Radopholus similis* Cobb), lesion (*Pratylenchus* spp), ring (*Criconemoides* spp), sting (*Belonolaimus longicaudatus* Rau) and several other plant parasitic nematodes.[7] An interesting observation was the apparent lack of any activity against the animal parasitic nematode *Nippostrongylus brasilense* Laue, and even the saprophagous nematode species *Caenorhabditis elegans* Maupas and *Panagrellus redivivus* (L) Goodey.

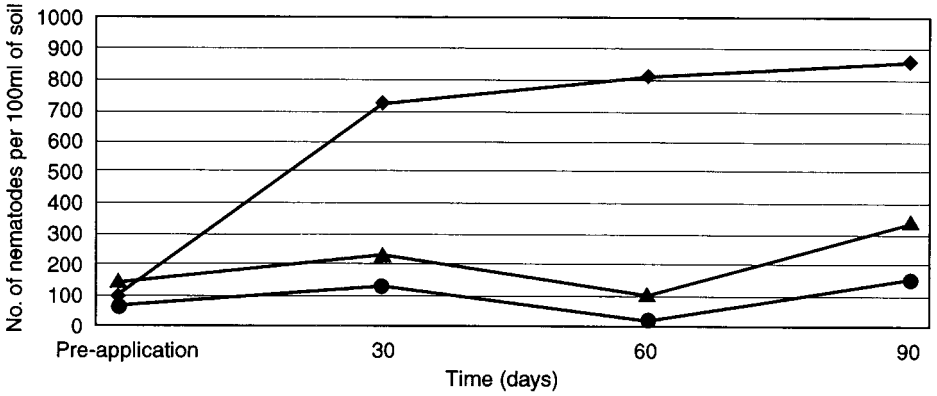

Figure 1. Numbers of root-knot nematodes in soil around table grape vines growing at Hermosillo, Mexico at 30-day intervals after treatment with (—●—) DiTera (25 litres ha^{-1}) or (—▲—) fenamiphos (Nemacur; 7 litres ha^{-1}); (—◆—) untreated. Mean of four replicates.

3 ACTIVE INGREDIENTS AND MODE OF ACTION

The nematicidal activity of DiTera is due primarily to the presence of many, relatively low-molecular-mass, water-soluble, compounds, which act synergistically. Fractionation and identification of specific, active, nematicidal molecules in DiTera is still in progress. Fermentation of *M verrucaria* in selected media results in the optimal production of nematode-specific active components. Several of the active principles have now been isolated, identified and are currently being evaluated for contact nematicidal response as well as other behavioral effects on nematodes.

In addition to contact nematicidal activity against juveniles of cyst nematodes, Twomey *et al*[8] recently reported that DiTera affects hatching of cyst nematode (*Globodera rostochiensis* Wollenw) eggs. These researchers did not observe any effects on the hatching of cysts, which typically is induced by hatching stimulants. However, exposure to DiTera seemed to affect the neurosensory responses of nematodes adversely, eventually affecting motility and host/mate finding behavior. The active ingredients also inhibited egg development. Recent studies have also confirmed the indirect effects of DiTera on the microbiology of the treated soils and the rhizosphere,[9] where it resulted in significant increases in antagonistic microorganisms capable of parasitizing nematode eggs; this effect was observed at all the rates and in the many soil types tested. While the specific reasons for such a biological response are not clearly known, it is believed that such an effect could account for the longer-term nematode control observed in commercial field situations.

4 COMMERCIAL PRODUCTION

DiTera is a product of controlled, sterile fermentation of a specific isolate of *M verrucaria* carried out in large fermentor vessels in media commonly used for microbial products; typical fermentations last for 72–96 hours, after which the mycelium is extracted and spray-dried as is the fermentation broth. The entire fermentation mass is utilized in the production of DiTera and the organism is killed during the processing steps, thus resulting in a 'killed-microbial' product. The nematicidal activity is thus presented in a natural active ingredient preparation defined as 'solids and solubles produced by the fermentation of the AARC-0255 isolate of *M verrucaria*.' This product is then formulated into different formulations, such as DiTera WDG (turf), DiTera G (banana) and DiTera ES (grapes, cole crops), using standard agricultural adjuvants to fit specific commercial markets.

One of the most important commercial attributes of DiTera is its relative safety *vis-a-vis* currently used chemical alternatives. Based on the acute toxicology battery, DiTera and its formulations have been classified as category III (slightly toxic) and IV (practically non-toxic) pesticides respectively. The non-target organism studies did not indicate any adverse effects against the avian or aquatic organisms tested.[7] The product and its formulations are exempted from the requirements of a tolerance on all agricultural commodities and have now been registered for commercial use in the USA under the microbial guidelines of the United States Environmental Protection Agency; several state and international registrations have also been secured for the granular and liquid formulations.

5 FIELD PERFORMANCE

During the past three years, development efforts on DiTera have been focused on field research on specific crops in many locations in the USA. Based on the specific nematicidal activity profile of the product, cyst nematodes (*Heterodera* spp) were considered the main target, particularly on cole crops in California. Early stage field evaluations in replicated, small plot trials using the liquid DiTera ES formulation showed significant reduction in cyst nematode population in cauliflower during the early, critical, stages of plant growth associated with enhancement of yield in the treated plots.[10] Larger-scale commercial trials on cauliflower, broccoli, grapes and bananas were conducted in 1996–98 in order to

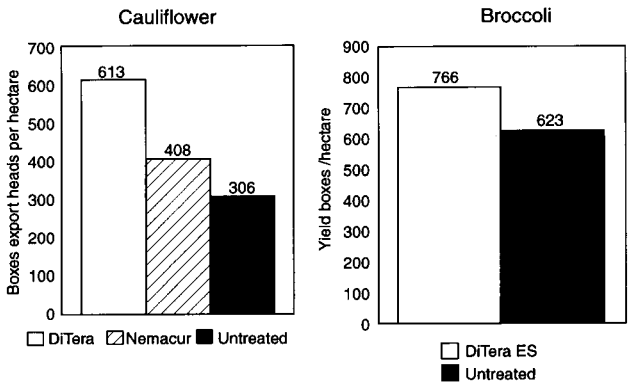

Figure 2. Yield benefits of treatment of cole crops at Salinas, California, USA against the sugar beet cyst nematode *Heterodera schachtii*. DiTera ES was applied as an 8″ band by sprinkler irrigation delivering 22 litres ha^{-1}. A. Cauliflowers; six replicate 0.3-ha plots per treatment. B. Broccoli; commercial trial involving four replicate 2 ha plots per treatment.

define the application parameters and to identify commercial benefits. Experimental details and the results are provided in the Figs 1 and 2.

6 CONCLUSIONS

The results demonstrate significant benefits in quantitative yield accompanied by nematode population reductions after application of DiTera treatments at critical stages in plant development. The product has now been commercialized in the cole crops and turf markets in the United States and the table grapes market in Mexico. Current development programs are focused towards addressing practical application parameters including timing, rates, methods of delivery, and new crops such as bananas, citrus and tobacco. Additionally, basic studies on the specific role of individual active molecules and the overall mechanisms involved in nematode management are under investigation. The unique product profile of DiTera offers an alternative, environmentally compatible option for plant parasitic nematode management in agricultural crops.

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The insecticidal activity of derivatives of the ionophore X-206

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Abstract: The naturally-occurring ionophore X206, originally isolated for its antibacterial activity, also exhibits broad insecticidal and acaricidal activity. This summary reports structure/activity studies with X206 and 34 derivatives as well as mode of action studies. Although many compounds showed promising insecticidal activity, it was only of a contact activity nature; furthermore, the acute LD_{50} of the compounds in rats precluded further development of these compounds.

Keywords: X206 derivatives; ionophore; insecticide; acaracide; mode of action

The ionophore X-206 (Fig 1, **1**) was isolated in the early 1950s,[1] and its X-ray structure was determined in 1975 (Fig 2).[2,3] X-206 was originally isolated for its antibacterial activity,[1] but in 1980 Chugai patented its insecticidal and acaricidal activities.[4] More recently Grafe and Schlegel[5] found a new organism which produced X-206 and its broad insecticidal and acaricidal activities were confirmed by the Novartis screening laboratories (Table 1)

Although an LD_{50} of 17 mg kg^{-1} for X-206 in mice was reported,[6] the insecticidal activity was so promising that a derivatisation program was started.[7] This programme was guided by the ion-complexing abilities of X-206. In order to kill insects it is important for X-206 derivatives to be able to bind ions and transport them across cell membranes.[8] This is reflected by the good correlation of the insecticidal activity with the complexing ability of the compounds (Table 1). Derivatives at the C(1)-, C(9)-, or C(34)- O-atoms showed a complete or almost complete lack of insecticidal activity. It is known from

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